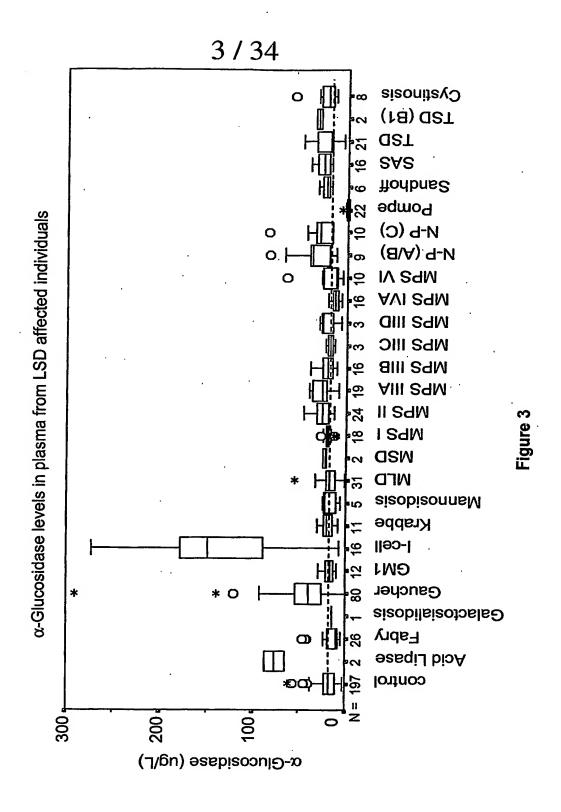
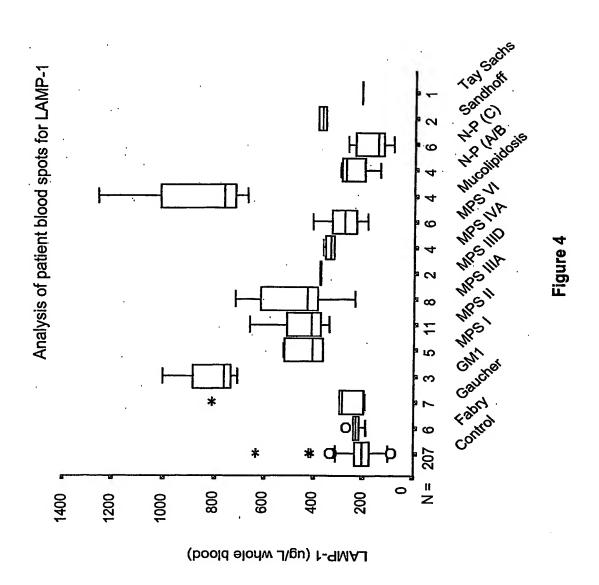


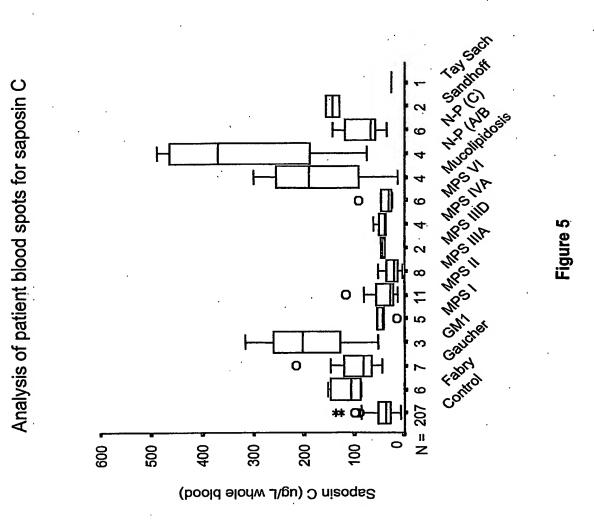
PCT/AU2004/000403



4./34



5/34



α-Glucosidase protein/activity determination in dried blood spots

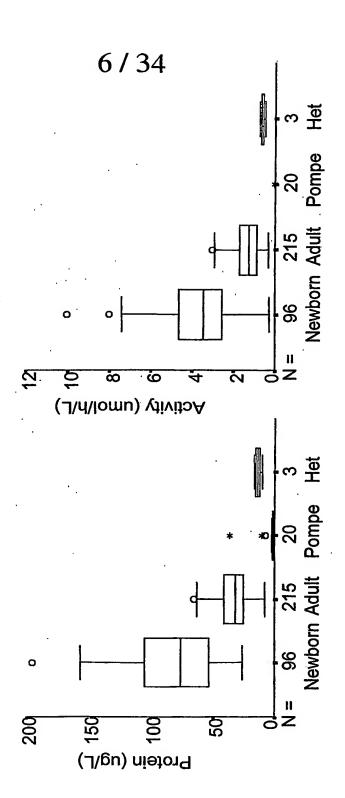
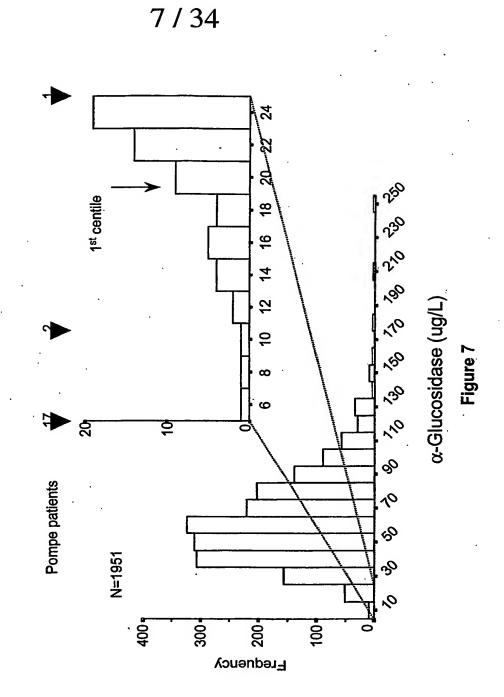


Figure (

α-Glucosidase protein distribution in neonates



8/34

LAMP-1 & saposin C newborn population distribution Dual TRFIMA assay for LAMP-1/saposin C

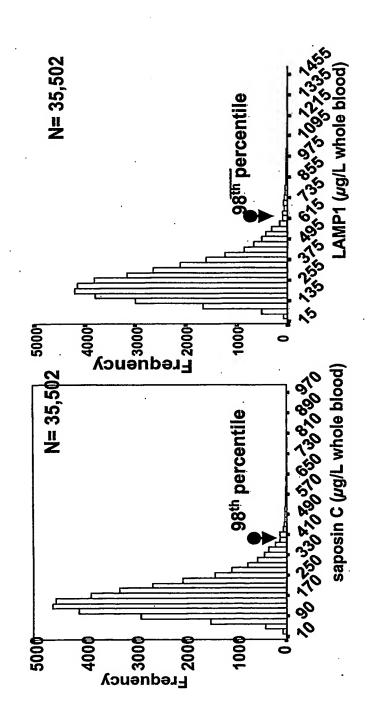
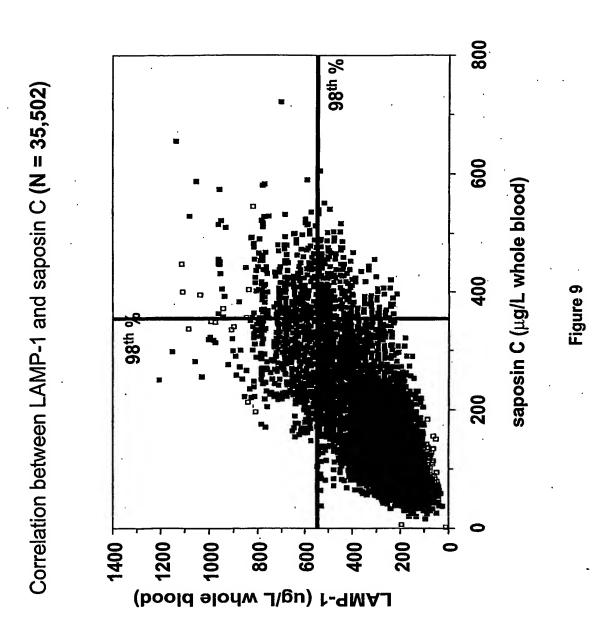


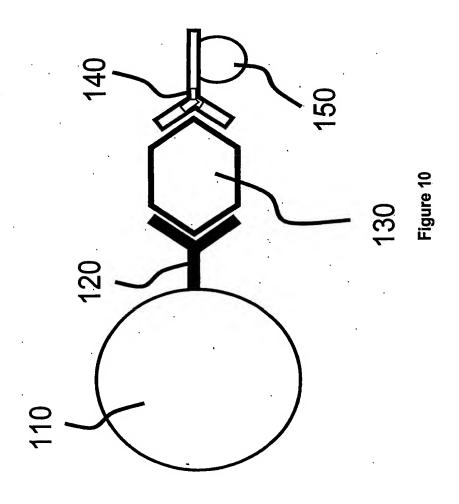
Figure 8

9/34

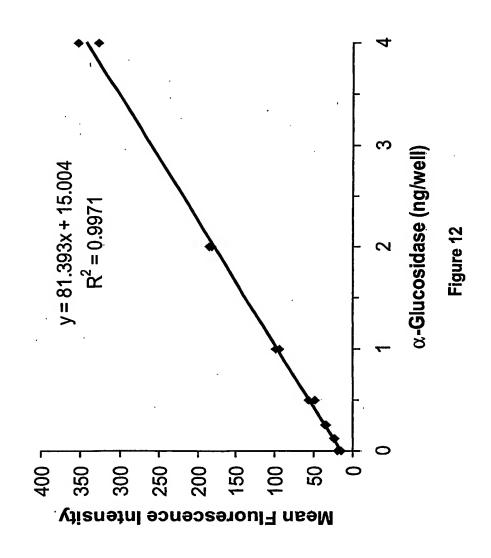


WO 2004/088322 PCT/AU2004/000403

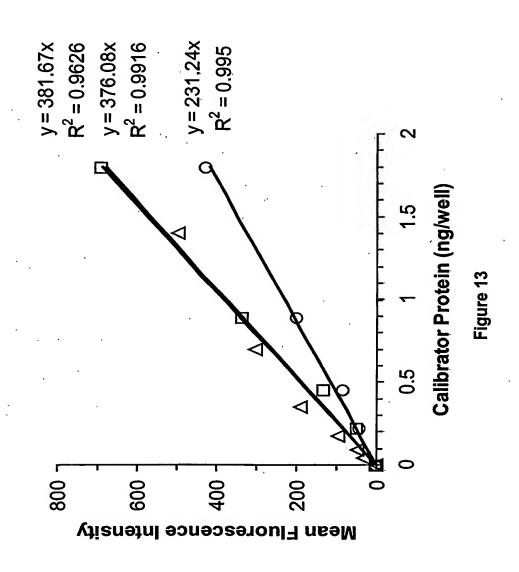
10/34



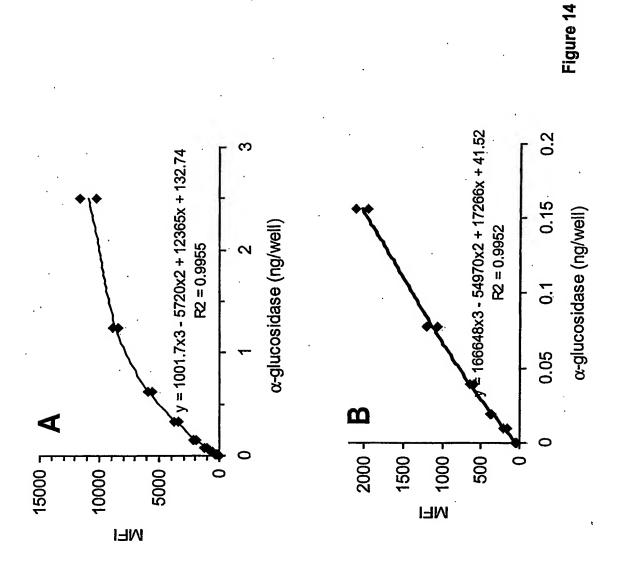
	iibody	Antibody reagents available for lysosomal proteins	for lysosol	mal prote	sins
Priority Disorder E	ш	Enzyme /Protein	Protein	Polycional	Monoclonal
3 20 20 20 20 20 20 20 20 20 20 20 20 20		Marker			(Complementary)
LAMP-1	LAMP-		CHO ex	Sheep	2
Saposin C	Saposir	<u>ر</u> د		Rabbit	2
CD 45	CD 45		commercial	,	
MPS I α-L-idur	α-L-idur	α-L-iduronidase	CHO ex	Sheep	_
Pompe disease α-glucosidase	α-gluco	sidase	CHO ex	Sheep	
Gaucher disease β-glucosidase	g-glucos	idase	commercial	Sheep	-
disease	α-galact	α-galactosidase A	commercial	Sheep	. 2
MPS VI N-acetylgalac	N-acetylg	N-acetylgalactosamine	CHO ex	Sheep	34
nn-Pick A/B	acid sohii	rsuipilatase acid sphindomvelinase	commercial	Sheen	•
	iduronate	iduronate-2-sulphatase	CHO ex	Sheep	ı
∀	galactose	galactose 6-sulphatase	CHO.ex	Rabbit	
MLD arylsulphatase A	arylsulph	atase A	CHO ex	Sheep	
disease	galactoce	galactocerebrosidase		•	
	heparan	heparan-N-sulphatase	CHO ex	Rabbit	
<u>8</u>	α-N-ace	α-N-acetylglucosaminidase	CHO ex	Rabbit	



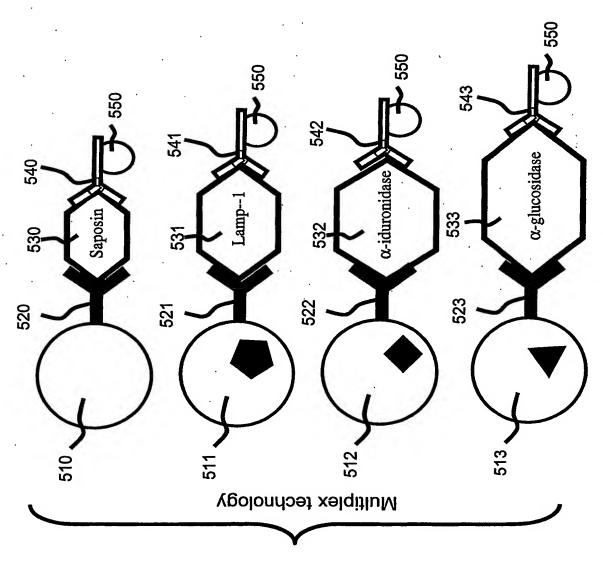
13 / 34



14/34

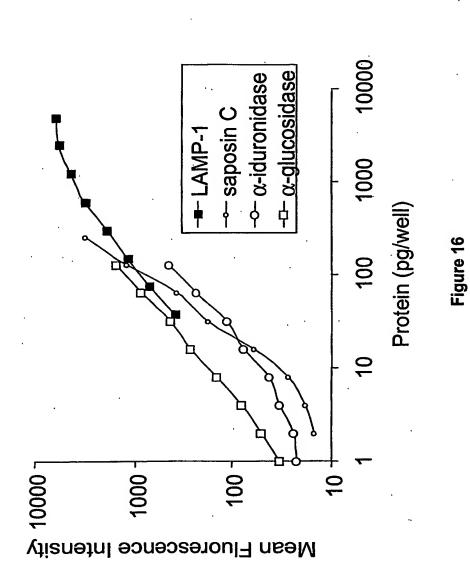


15 / 34

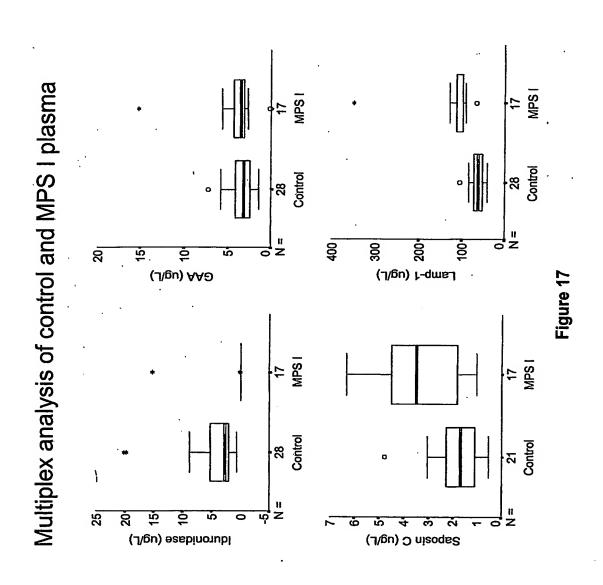


xəlq-₽

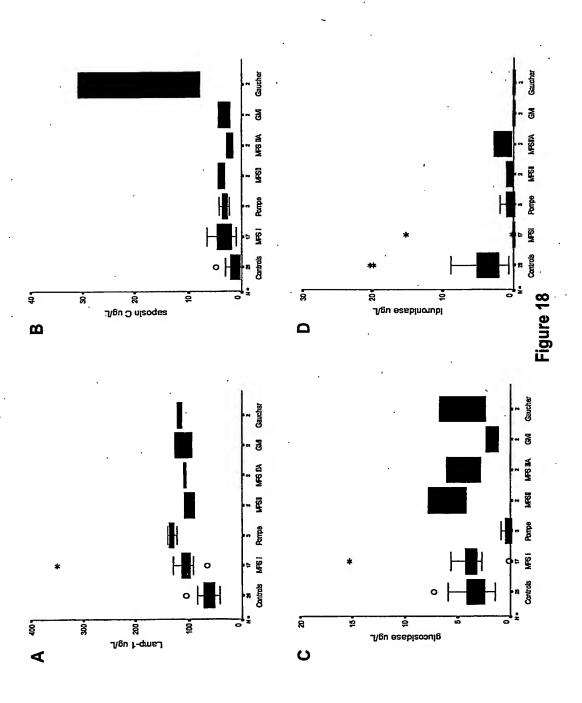
Multiplex calibration curves: 4-plex



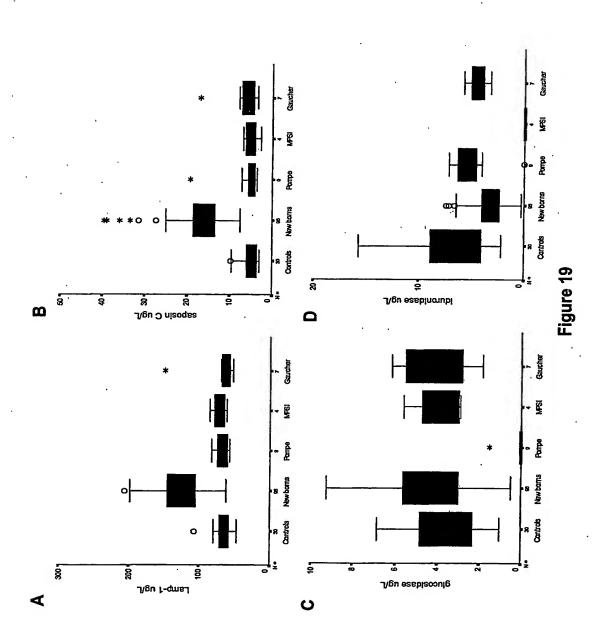
17/34



18/34



19 / 34



20/34

Protein markers for 7-Plex LSD screening

().
2	=
ğ	3
Š	Ź.
C	Š
2	2
	3
7	
Š	=
٩	7

Disorder	Enzyme Deficiency	Australian Prevalence	Therapy
Gaucher disease Fabry disease MPS I Pompe disease MPS VI	β-glucosidase α-galactosidase A α-L-iduronidase α-glucosidase N-acetylgalactosamine 4-sulphatase	1 in 57,000 1 in 117,000 1 in 88,000 1 in 146,000 1 in 235,000	ERT / BMT ERT ERT / BMT ERT (trials) BMT / ERT (trials)

Figure 20

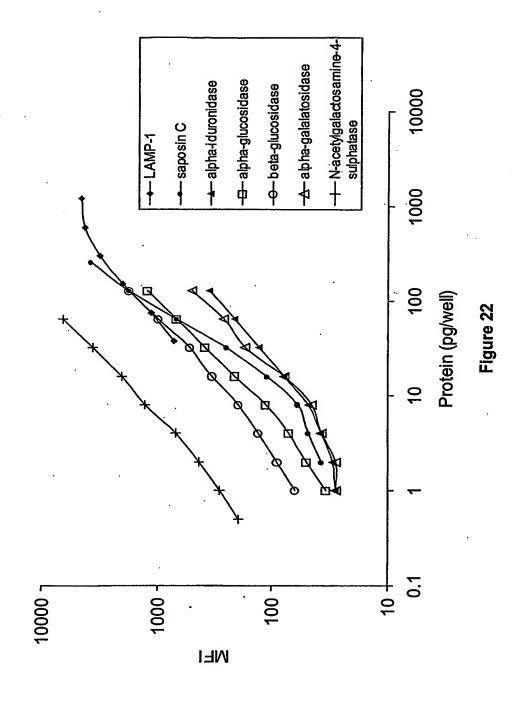
Most LSD patients have reduced protein. Total prevalence detected with 7-plex is 1 in 20,600.

21/34

Antibody reagents used in 7-plex assays

ng/well	16	8	91	91	32	32	32
Reporter - antibody	Sheep anti Lamp-1 polyclonal	Monoclonal S13C1 G2 G3	Monoclonal 43D1	Monoclonal Id1A	Monoclonal AG2.6F5.1151	Sheep anti β-glucosidase polyclonal	Sheep anti 4-sulphatase polyclonal
μg/1.25e6 beads	6	6	5	98	6	6	6
Capture antibody	Sheep anti Lamp-1 polyclonal	Monoclonal 7B2	Sheep anti α-glucosidase polyclonal	Sheep anti œ-iduronidase polyclonal	Monoclonal AG2.GG9.6.1.6	Sheep anti β-glucosidase polycional	Sheep anti 4-sulphatase polyclonal
Bead region	22	42	26	24	43	45	. 94
Assay	Lamp-1	Saposin C	α-glucosidase	α-iduronidase	α-galactosidase	β-glucosidase	N-acetyl- galactosamine-4- sulphatase

22 / 34



23 / 34

LAMP-1							
	Saposin C	α-iduronidase	α-glucosidase	β- glucosidase	α-galacto sidase	N-acetyl galactosamine- 4-sufohatase	
Jui/gu	ng/mL	ng/mL	ng/mL	ng/mL	Jm/gu	ng/mL	
32.9	12.7	5.0	7.7	3.3	4.6	6.0	
36.4	10.7	4.8	4.0	3.0	4.2	1.5	
34.5	8.9	7.5	8.2	3.5	4.6	1.2	
28.7	13.2	10.0	8.8	4.1	7.4	1.7	
36.5	14.0	6.3	9.2	3.0	5.9	6:0	
38.5	22.3	7.9	14.0	6,4	8.5	2.3	
38.8	13.5	11.2	10.8	5.3	4.6	1.5	
31.0	12.0	4.9	9.4	3.5	2.7	1.3	
34.7	13.0	8.2	5.6	4,4	4.2	1.1	
29.1	12.1	4.3	5.3	4.0	5.1	1.1	
36.2	13.0	5.9	6.9	3.7	3.6	1.7	
40.9	17.6	8,5	10.1	5.5	7.2	1.8	
34.9	13.6	7.0	8.3	4.1	5.2	1.4	
3.8	3.4	2.2	2.7	11	1.7	0.4	
28.7	8.9	4.3	4.0	3.0	2.7	0.0	
40.9	22.3	11.2	14.0	6.4	8.5	2.3	
0.1	0.3	0.3	0.3	0.3	0.3	0.3	
8:0	0.7	9.0	0.5	0.7	0.5	90	
1.2	1.6	1.6	1.7	1.5	1.6	97	
	38.5 38.5 38.5 38.8 34.7 29.1 29.1 29.1 29.1 29.1 29.1 29.1 29.1		13.0 13.0 13.0 13.0 13.0 13.6 13.6 13.6 22.3 0.3 0.7	14.0 6.3 12.3 7.9 13.5 11.2 12.0 4.9 13.0 8.2 13.0 8.2 13.0 5.9 17.6 8.5 13.6 7.0 3.4 2.2 8.9 4.3 22.3 11.2 0.3 0.3 0.7 0.6	14.0 6.3 9.2 14.0 6.3 9.2 22.3 7.9 14.0 13.5 11.2 10.8 12.0 4.9 9.4 13.0 8.2 5.3 13.1 4.3 5.3 13.6 7.0 8.3 17.6 8.5 10.1 13.6 7.0 8.3 13.4 2.2 2.7 8.9 4.3 4.0 22.3 11.2 14.0 0.7 0.6 0.5 0.7 0.6 0.5 1.6 1.7	14.0 6.3 9.2 4.1 14.0 6.3 9.2 3.0 22.3 7.9 14.0 6.4 13.2 4.9 9.4 3.5 12.0 4.9 9.4 3.5 13.0 8.2 5.3 4.0 13.1 4.3 5.3 4.0 13.6 7.0 8.3 4.1 13.6 7.0 8.3 4.1 13.6 7.0 8.3 4.1 8.9 4.3 4.0 3.0 22.3 11.2 14.0 6.4 0.7 0.6 0.5 0.7 1.6 1.6 1.7 1.5	140 6.3 9.2 3.0 5.9 140 6.3 9.2 3.0 5.9 22.3 7.9 14.0 6.4 8.5 12.3 11.2 10.8 5.3 4.6 12.0 4.9 9.4 3.5 2.7 12.0 4.9 9.4 3.5 2.7 12.1 4.3 5.6 4.4 4.2 13.0 8.2 5.6 4.4 4.2 13.0 5.9 6.9 3.7 3.6 17.6 8.5 10.1 5.5 7.2 13.6 7.0 8.3 4.1 5.2 13.6 7.0 8.3 4.1 5.2 22.3 11.2 14.0 6.4 8.5 0.3 0.3 0.3 0.3 0.3 0.7 0.6 0.5 0.7 0.5 1.6 1.7 1.7 1.6 1.6

Figure 23

Age LAMP-1 Saposin C C-ciduronidase α-galucosidase β-glucosidase α-case α-case supfant α-ciduronidase α-case α-ciduronidase α-cidurosidase α-cidurosidase <t< th=""><th></th><th></th><th>-</th><th>-</th><th></th><th></th><th></th><th></th><th></th></t<>			-	-					
D years ng/mL ng/		Age	LAMP-1	Saposin C	α-iduronidase	α-glucosidase			galactosamine-4 sulphatase
233 41 14 11 15 10 249 39 18 14 13 13 721 367 40 37 27 65 721 52 13 27 13 13 600 366 09 102 41 13 14 600 366 09 102 41 178 64 600 366 09 102 41 178 66 702 303 18 66 37 64 10 702 439 41 10 08 10 10 11 11 11 11 10	Sample ID	years	ng/mL	ng/mL	ng/mL	ng/mL	ng/mľ.	ng/mL	ng/mľ.
651 367 40 39 18 14 113 14 10 13 10 10 13 10 13 13 13 13 13 13 13 13 10 10 13 10 10 13 10 10 13 10 10 10 10 10 10 10 10 10 10 10 10 11 13 10	Newborn 1		23.3	41	14	11	15	9	0.2
65.1 36.7 4.0 3.7 2.7 65. 24.1 58.2 1.7 121 5.2 135 62.0 36.4 2.1 1.25 4.5 195 60.0 36.6 0.9 10.2 4.1 17.8 60.0 36.6 0.9 10.2 4.1 17.8 60.1 3.7 1.4 10.0 0.8 10 7.5 4.2 1.8 6.6 3.1 6.6 9. 4.2 1.8 6.6 3.1 6.6 1. 3.0 1.8 1.1 1.0 0.9 2. 5.2 4.1 5.4 3.9 5.0 14.4 3. 4.1 1.4 6.6 3.0 1.0 5.2 5.2 4. 5. 4.1 1.4 4.1 1.0 5.2 5.2 1.0 5.2 5.2 1.0 5.2 5.2 1.0 5.2 5.2 <	Newborn 2		24.9	3.0	18	14	13	13	0.2
72.1 88.2 1.7 12.1 5.2 13.5 69.0 36.4 2.1 12.5 4.5 9.6 69.0 36.6 0.9 10.2 4.1 17.8 44.7 30.3 1.8 6.6 3.7 6.4 20.0 3.9 4.1 1.0 0.8 1.0 20.0 3.9 4.1 9.4 3.9 6.6 30.2 4.1 1.0 0.8 1.0 0.8 30.2 4.2 1.8 4.1 1.2 5.6 1.5 1.2 1.2 1.2 1.2 1.2 1.2 1.2 1.2 1.2 1.2 1.2 1.2 1.2 1.2 1.2 1.2<	Newborn 3		651	367	40	3.7	2.7	6.5	22
690 364 2.1 125 45 96 690 366 09 102 41 178 447 303 18 66 41 178 191 3.7 14 10 08 10 205 3.9 18 10 08 10 206 43.9 41 94 39 66 36.8 341 14 66 29 58 36.8 341 14 66 29 58 36.8 341 14 66 29 58 36.9 341 14 66 29 58 37 14 14 56 29 58 4 70.5 54 129 14 44 150 5 64.8 30 13 41 44 10 6.0 69.1 61.6 57 10 45 10	Newborn 4		72.1	58.2	17	12.1	5.2	13.5	22
bom f 69.0 36.6 0.9 10.2 4.1 17.8 bom 7 44.7 30.3 1.8 6.6 3.7 6.4 bom 8 19.1 3.7 1.4 1.0 0.8 1.0 bom 9 42.5 43.9 4.1 9.4 3.9 6.6 bom 10 5.6 5.4 2.9 1.8 1.1 0.9 bom 11 36.8 34.1 1.4 6.6 2.9 5.8 bom 12 36.8 34.1 1.4 6.6 2.9 5.8 bom 13 56.2 64.4 2.9 1.16 5.9 5.8 bom 14 8.1 1.4 9.4 3.9 5.6 1.4 bom 15 8.2 6.4 2.9 1.1 1.1 1.4 bom 16 6.3 3.2 3.2 1.1 4.5 1.1 bom 17 6.0 4.2 3.4 3.1 4.5 1.1	Newhorn 5		542	564	2.1	12.5	4.5	9.6	28
bom 8 191 37 64 37 64 bom 8 191 37 14 10 08 10 bom 9 425 198 33 38 31 55 bom 10 526 439 41 94 39 66 bom 11 209 39 18 11 11 09 bom 12 368 341 18 11 10 09 bom 12 362 644 29 136 56 159 50 bom 13 562 644 29 136 50 144 10 09 50 144 10 09 50 144 10 09 50 144 10 09 50 144 10 09 50 144 10 09 10 10 10 10 10 10 10 10 10 10 10 10 10 10 <td< td=""><td><u> Newborn 6</u></td><td></td><td>069</td><td>36.6</td><td>0.0</td><td>10.2</td><td>4.1</td><td>17.8</td><td>2.0</td></td<>	<u> Newborn 6</u>		069	36.6	0.0	10.2	4.1	17.8	2.0
born 8 191 37 14 10 08 10 born 9 425 198 33 38 31 55 born 10 226 439 41 94 39 66 born 11 368 341 14 66 29 58 born 12 368 341 14 66 29 58 born 12 362 644 29 136 56 150 born 13 562 644 29 136 56 150 born 14 31 42 28 14 18 66 born 15 81 44 29 143 56 143 born 16 83 34 30 128 57 143 born 26 63 41 30 44 140 45 140 born 27 648 34 36 31 46 111 born 28	Newhorn 7		. 447	303	18	99	3.7	8,9	3.5
com 9 47.5 19.8 3.3 3.8 3.1 5.5 com 10 \$2.6 43.9 4.1 9.4 3.9 6.6 com 11 \$2.9 3.9 1.8 1.1 1.1 0.9 com 12 \$6.8 3.41 1.4 6.6 2.9 5.8 com 12 \$6.2 \$4.4 2.9 1.29 \$5.0 14.4 com 13 \$6.2 \$6.2 \$6.2 1.29 \$6.0 14.4 com 14 \$6.2 \$6.2 \$1.2 \$6.0 14.4 com 15 \$6.2 \$6.2 \$1.2 \$6.0 14.4 com 15 \$6.2 \$6.2 \$1.0 \$1.0 \$1.0 com 16 \$6.3 \$6.4 \$1.0 \$1.0 \$1.0 \$1.0 com 17 \$6.4 \$1.2 \$1.0 \$1.0 \$1.0 \$1.0 \$1.0 \$1.0 \$1.0 \$1.0 \$1.0 \$1.0 \$1.0 \$1.0 \$1.0 \$1.0	Newhorn 8		19.1	3.7	1.4	1.0	0.8	9	0.2
pom 10 \$2.6 43.9 4.1 9.4 3.9 6.6 pom 11 20.9 3.9 1.8 1.1 1.1 0.9 pom 12 36.8 3.4 1.4 6.6 2.9 5.8 pom 12 56.2 6.4 2.9 13.6 5.9 18.9 pom 14 70.5 5.7 2.8 12.9 5.0 14.4 pom 15 81.1 45.6 1.6 8.3 4.1 18.5 pom 15 83.7 7.2 2.6 13.3 6.4 29.0 pom 15 83.7 7.2 2.6 13.3 6.4 29.0 pom 16 82.4 67.8 3.0 9.4 5.7 14.0 pom 27 60.1 5.7 2.1 4.2 14.6 pom 18 82.4 64.5 3.4 16.7 4.2 2.2 pom 28 60.7 33.8 3.5 10.0 3.6 4.1 1.2 </td <td>Newhorn 9</td> <td></td> <td>42.5</td> <td>19.8</td> <td>3.3</td> <td>3.8</td> <td>3.1</td> <td>33</td> <td>16</td>	Newhorn 9		42.5	19.8	3.3	3.8	3.1	33	16
bom 11 20.9 3.9 1.8 1.1 1.1 0.9 bom 12 36.8 34.1 1.4 66. 2.9 5.8 bom 13 56.2 64.4 2.9 13.6 5.0 15.9 bom 14 70.5 55.7 2.8 12.9 5.0 14.4 bom 15 81.1 45.6 1.6 2.9 14.4 18.5 bom 15 81.1 45.6 1.6 1.2 5.0 14.4 bom 16 6.3 5.48 3.0 1.2.8 5.7 14.3 bom 17 83.7 7.2 2.6 13.3 6.4 1.4 bom 18 82.4 67.8 3.9 9.4 5.7 14.3 bom 28 68.4 3.9 9.4 5.7 14.3 bom 29 68.1 3.4 10.0 4.6 10.0 bom 24 60.2 3.4 3.1 4.6 10.0 bom 25	Newhorn 10		52.6	43.9	4.1	9.4	3.9	99	2.4
bom 12 368 341 14 66 29 58 bom 13 \$62 644 29 136 56 159 bom 14 70.5 55.7 28 129 50 144 bom 15 81.1 456 16 83 41 185 bom 15 81.1 456 16 128 57 126 bom 16 63.9 548 30 94 57 143 bom 17 824 67.8 34 36 66 710 bom 20 69.1 61.6 57 143 86 143 bom 21 60.2 43.3 29 44 166 111 bom 21 60.2 43.3 29 71 46 111 bom 22 63.2 83.9 56 167 64 166 bom 23 60.2 43.3 2.9 71 46 111 bom 24 <td>Newhorn 11</td> <td></td> <td>200</td> <td>30</td> <td>18</td> <td>11</td> <td>11</td> <td>00</td> <td>0.2</td>	Newhorn 11		200	30	18	11	11	00	0.2
bom 13 562 644 29 136 56 150 bom 14 705 557 28 120 50 144 bom 15 811 456 16 83 41 185 bom 16 639 548 30 128 57 126 bom 17 837 722 26 133 64 290 bom 17 837 722 26 133 64 290 bom 18 648 584 39 94 57 143 bom 20 691 616 57 210 65 146 bom 20 691 41 45 110 42 146 bom 21 602 433 29 71 46 111 bom 24 607 31 32 100 36 100 bom 25 824 64.5 34 101 54 20 bom 26	Newhorn 12		368	34.1	1.4	99	2.9	\$3	27
bom 14 70.5 55.7 2.8 17.9 5.0 14.4 bom 15 81.1 45.6 1.6 8.3 4.1 18.5 bom 16 63.9 54.8 3.0 12.8 5.7 12.6 bom 17 83.7 72.2 2.6 13.3 6.4 29.0 bom 17 83.7 72.2 2.6 13.3 6.4 29.0 bom 18 64.8 58.4 3.9 9.4 5.7 14.3 bom 20 69.1 61.6 5.7 21.0 6.5 14.6 bom 20 60.2 43.3 2.9 7.1 4.6 11.1 bom 24 60.2 43.3 2.9 7.1 4.6 11.1 bom 25 82.4 64.5 3.4 10.1 5.4 2.0 bom 26 82.4 64.5 3.4 10.1 5.4 2.0 bom 27 60.7 3.1 3.2 1.0 3.6	Newborn 13		562	64.4	2.9	13.6	5.6	159	3.8
bom 15 811 456 16 83 41 185 bom 16 639 548 30 128 57 126 bom 17 837 722 26 133 64 290 bom 18 648 584 39 94 57 143 bom 19 824 678 34 96 66 710 bom 20 691 616 57 110 65 146 bom 21 472 242 36 91 31 80 bom 22 602 433 29 71 46 166 bom 23 602 433 29 71 46 109 bom 24 707 317 27 119 42 224 bom 25 607 321 35 100 36 41 172 bom 25 607 32 34 101 46 160 v	Newhorn 14		70.5	55.7	2.8	12.0	5.0	14.4	27
bom 16 63.9 54.8 3.0 12.8 5.7 12.6 bom 17 83.7 72.2 2.6 13.3 6.4 29.0 bom 18 64.8 58.4 3.9 9.4 5.7 14.3 bom 19 82.4 67.8 3.4 9.6 6.6 71.0 bom 20 69.1 61.6 5.7 21.0 6.5 14.6 bom 20 69.1 61.6 5.7 21.0 6.5 14.6 bom 21 47.2 24.2 3.6 9.1 3.1 8.0 bom 21 47.2 24.2 3.6 16.7 6.4 16.6 bom 22 43.2 2.9 7.1 4.6 11.1 bom 24 60.7 32.1 3.5 10.0 3.6 10.9 bom 25 60.7 32.1 3.4 4.0 1.7 2.9 bom 26 58.2 42.9 2.8 9.4 4.6 16.0 <td>Newborn 15</td> <td></td> <td>811</td> <td>456</td> <td>1.6</td> <td>8.3</td> <td>41</td> <td>18.5</td> <td>1.9</td>	Newborn 15		811	456	1.6	8.3	41	18.5	1.9
born 17 83.7 72.2 2.6 13.3 6.4 29.0 born 18 64.8 58.4 3.9 9.4 5.7 14.3 born 19 82.4 67.8 3.4 9.6 6.6 71.0 born 20 69.1 61.6 5.7 21.0 6.5 14.6 born 20 69.1 61.6 5.7 21.0 6.5 14.6 born 21 63.2 83.9 5.6 16.7 6.4 16.6 born 21 60.2 43.3 2.9 7.1 4.6 11.1 born 22 83.9 5.6 10.0 3.6 10.9 born 24 70.7 31.7 2.7 11.9 4.2 2.2 born 24 70.7 31.7 2.7 11.9 4.2 2.2 born 25 82.4 64.5 3.4 10.1 5.4 2.6 born 28 86.5 7.4 2.2 18.8 16.4 7.2<	Newborn 16		639	548	30	12.8	5.7	12.6	25
born 18 648 584 39 94 57 143 born 19 824 678 34 96 66 710 born 20 691 616 57 210 655 146 born 21 472 242 36 91 31 80 born 21 632 83.9 56 167 64 166 born 22 602 43.3 29 71 46 111 born 23 602 43.3 29 71 46 111 born 24 707 317 27 119 42 224 born 25 82.4 64.5 3.4 101 54 269 born 26 7.4 2.2 100 3.6 4.1 127 born 27 86.5 74.4 2.2 188 164 7.2 v 196 2.2 2.8 9.4 4.6 16.0 v </td <td>Newborn 17</td> <td></td> <td>83.7</td> <td>72.2</td> <td>. 26</td> <td>13.3</td> <td>6.4</td> <td>29.0</td> <td>33</td>	Newborn 17		83.7	72.2	. 26	13.3	6.4	29.0	33
born 19 82.4 67.8 3.4 9.6 6.5 71.0 born 20 69.1 61.6 5.7 21.0 65.5 14.6 born 21 47.2 24.2 3.6 9.1 3.1 8.0 born 22 63.2 83.9 5.6 16.7 6.4 16.6 born 23 70.7 31.7 2.7 11.9 4.2 2.2 born 24 70.7 31.7 2.7 11.9 4.2 2.2 born 25 60.7 31.7 2.7 11.9 4.2 2.2 born 26 62.7 35.8 3.5 10.0 3.6 10.9 born 27 86.7 3.4 10.1 5.4 2.2 2.4 born 28 86.5 74.4 2.2 18.8 16.4 17.3 syc 42.9 2.8 2.8 2.9 4.6 16.0 y 19.1 3.7 0.9 1.0 0.9	Newborn 18		64.8	58.4	3.9	9.4	5.7	143	4.8
born 20 69.1 61.6 57 21.0 65 146 born 21 47.2 24.2 3.6 9.1 3.1 8.0 born 21 60.2 43.3 2.9 7.1 4.6 11.1 born 22 60.2 43.3 2.9 7.1 4.6 11.1 born 24 70.7 31.7 2.7 11.9 4.2 2.24 born 24 60.7 31.7 2.7 11.9 4.2 2.24 born 25 82.4 64.5 3.4 10.1 5.4 2.2 born 26 82.4 64.5 3.4 10.1 5.4 2.6 born 27 86.5 74.4 2.2 18.8 16.4 7.2 syc 42.9 2.8 9.4 4.6 16.0 v 19.6 2.2 2.9 1.73 v 19.1 3.7 0.9 1.0 v 19.4 2.2 1.0	Newborn 19		82.4	879	3.4	9.6	99	710	33
born 21 472 242 36 91 31 80 born 22 632 83.9 56 167 64 166 born 23 602 43.3 2.9 71 4.6 111 born 24 70.7 31.7 2.7 11.9 4.6 11.1 born 25 62.7 35.8 3.5 10.0 3.6 10.9 born 26 82.4 64.5 3.4 10.1 5.4 2.2 born 27 60.7 32.1 3.2 9.6 4.1 12.7 born 28 86.5 74.4 2.2 18.8 16.4 7.2 syc 196 2.2 2.8 9.4 4.6 16.0 v 196 2.2 1.2 2.9 17.3 v 191 3.7 0.9 1.0 0.9 v 191 3.7 0.9 1.0 0.9 v 10.1 0.3	Newborn 20		691	919	. 57	210	6.5	146	3.7
born 22 632 83.9 56 167 64 166 born 24 60.2 43.3 2.9 7.1 4.6 11.1 born 24 70.7 31.7 2.7 11.9 4.2 22.4 born 25 62.7 35.8 3.5 10.0 3.6 10.9 born 26 82.4 64.5 3.4 10.1 5.4 26.9 born 27 80.7 3.21 3.2 9.6 4.1 12.7 born 28 86.5 74.4 2.2 18.8 16.4 72.2 born 28 86.5 74.4 2.2 18.8 16.4 72.2 w 196 22.8 1.2 5.1 2.9 17.3 w 191 3.7 0.9 1.0 0.8 0.9 w 191 3.7 0.9 0.6 1.1 W 0.3 0.1 0.5 0.6 1.1 W 0	Newborn 21		47.2	242	3.6	9.1	3.1	80	15
born 23 60.2 43.3 2.9 7.1 4.6 11.1 born 24 70.7 31.7 2.7 11.9 4.2 22.4 born 25 62.7 35.8 3.5 10.0 3.6 10.9 born 26 82.4 64.5 3.4 10.1 5.4 26.9 born 27 80.7 3.1 3.2 9.6 4.1 12.7 born 27 86.5 74.4 2.2 18.8 16.4 72.2 w 19.6 22.8 1.2 9.4 4.6 16.0 v 19.6 22.8 1.2 5.1 2.9 1.73 w 19.1 3.7 0.9 1.0 0.8 0.9 w 19.1 3.7 0.9 1.0 0.8 0.9 w 19.1 3.7 0.9 0.6 1.1 w 10.0 0.3 0.1 0.5 0.6 1.1 MOM	Newborn 22		63.2	839	5.6	16.7	6.4	166	48
born 24 70.7 31.7 2.7 11.9 4.2 2.24 born 25 62.7 35.8 3.5 10.0 3.6 10.9 born 26 82.4 64.5 3.4 10.1 5.4 26.9 born 27 60.7 32.1 3.2 9.6 4.1 12.7 born 27 86.5 74.4 2.2 18.8 16.4 77.2 sge 58.2 42.9 2.8 9.4 4.6 16.0 v 191 3.7 0.9 1.0 0.8 0.9 v 191 3.7 0.9 1.0 0.8 0.9 v 191 3.7 0.9 1.0 1.6 1.1 v 191 3.7 0.9 1.0 0.6 1.1 v 1.0 0.3 0.1 0.3 0.1 0.2 0.1 0.1 fwOM 1.5 2.0 2.0 2.2 3.6	Newborn 23		602	43.3	2.9	7.1	46	111	3.0
born 25 62.7 35.8 3.5 10.0 3.6 10.9 born 26 82.4 64.5 3.4 10.1 5.4 26.9 born 27 60.7 32.1 3.2 9.6 4.1 12.7 born 28 86.5 74.4 2.2 18.8 16.4 77.2 sge 58.2 42.9 2.8 9.4 4.6 16.0 v 191 3.7 0.9 1.0 0.8 0.9 v 191 3.7 0.9 1.0 0.8 0.9 w 191 3.7 0.9 1.0 1.6 7.2 w 191 3.7 0.9 1.0 0.8 0.9 w 0.3 0.1 0.3 0.1 0.2 0.1 0.1 MOM) 1.5 2.0 2.0 2.2 3.6 4.5	Newborn 24		70.7	31.7	2.7	119	42	22.4	21
born 26 82.4 64.5 3.4 10.1 5.4 26.9 born 27 60.7 32.1 3.2 9.6 4.1 12.7 born 28 86.5 74.4 2.2 18.8 16.4 77.2 age 58.2 42.9 2.8 9.4 4.6 16.0 v 19.6 27.8 1.2 5.1 2.9 17.3 v 19.1 3.7 0.9 1.0 0.8 0.9 v 19.1 3.7 0.9 1.0 0.8 0.9 w 0.3 0.5 0.4 0.5 0.6 1.1 (MOM) 0.3 0.1 0.3 0.1 0.2 0.2 0.1 0.1	Newborn 25		62.7	358	35	10.0	3.6	10.9	25 .
born 27 60.7 32.1 3.2 96 4.1 12.7 born 28 86.5 74.4 2.2 18.8 16.4 77.2 age 58.2 42.9 2.8 9.4 4.6 16.0 v 19.6 22.8 1.2 5.1 2.9 17.3 v 19.1 3.7 0.9 1.0 0.8 0.9 v 19.1 3.7 0.9 1.0 0.8 0.9 MOOM) 0.3 0.1 0.3 0.1 0.2 0.1 0.1 MOOM) 1.5 2.0 2.0 2.2 3.6 4.5	Newborn 26		82.4	64.5	3.4	10.1	5.4	269	33
born 28 86.5 74.4 2.2 18.8 16.4 77.2 age 58.2 42.9 2.8 9.4 4.6 16.0 v 196 27.8 1.2 5.1 2.9 17.3 v 191 3.7 0.9 1.0 0.8 0.9 v 191 3.7 0.9 1.0 0.8 0.9 v 0.3 0.5 0.4 0.5 0.6 1.1 OMOM) 0.3 0.1 0.3 0.1 0.2 0.1 MOMIN 1.5 2.0 2.0 2.2 3.6 4.5	Newborn 27		60.7	32.1	3.2	96	4.1	12.7	19
upper 582 429 28 94 4.6 160 v 196 228 12 51 29 173 v 191 37 0.9 1.0 0.8 0.9 v(MOM) 0.3 0.1 0.3 0.1 0.5 0.6 1.1 (MOM) 0.5 2.0 2.0 2.2 3.6 4.5	Newborn 28		865	74.4	22	18.8	16.4	72.2	. 47
W 196 228 12 51 29 173 191 37 0.9 1.0 0.8 0.9 W(MOM) 0.3 0.5 0.4 0.5 0.6 1.1 (MOM) 0.3 0.1 0.3 0.1 0.2 0.1 0.1 (MOM) 1.5 2.0 2.0 2.2 3.6 4.5	Average		582	429	28	9.4	4.6	160	2.5
(MOM) 1.5 1.9 0.9 1.0 0.8 0.9 (MOM) 0.3 0.1 0.9 1.0 0.8 0.9 (MOM) 0.3 0.1 0.3 0.1 0.5 0.6 1.1 (MOM) 1.5 2.0 2.0 2.2 3.6 4.5	StDev		19.6	22.8	12	5.1	2.9	173	13
(MOM) 86.5 83.9 5.7 21.0 164 72.2 (MOM) 0.3 0.1 0.4 0.5 0.6 1.1 (MOM) 0.3 0.1 0.3 0.1 0.2 0.1 (MOM) 1.5 2.0 2.0 2.2 3.6 4.5	Min		19.1	37	00	1.0	0.8	90	0.2
0 0.3 0.5 0.4 0.5 0.6 1.1 0.3 0.1 0.3 0.1 0.2 0.1 1.5 2.0 2.0 2.2 3.6 4.5	Max		865	839	5.7	21.0	16.4	72.2	4.8
0.3 0.1 0.3 0.1 0.2 0.1 1.5 2.0 2.0 2.2 3.6 4.5	StDev(MOM)		0.3	0.5	0.4	0.5	9.0	1.1	0.5
1.5 2.0 2.0 2.2 3.6 4.5	Min (MOM)		0.3	0.1	0.3	0.1	0.2	0.1	0.1
	Max (MOM)		1.5	2.0	2.0	2.2	3.6	4.5	1.9

igure 24

Newborn control protein values

25 / 34

Pearson correlation	ion coefficients	s for protein n	coefficients for protein markers in dried blood spots from newborns.	d blood spots fr	om newborns.		
	LAMP-1	Saposin C	α- iduronidase	α- glucosidase	β- glucosidase	α- galactosidase	N- acetylgalactosamine 4-sulphatase
LAMP-1	1.00	0.82ª	0.31	0.73 a	0.70ª	0,69 ⁸	0.68ª
Saposin C	0.82 a	1.00	0.47	0.85 a	0.75ª	0.61 a	0.88 8
α-iduronidase	0.31	0.47b	1.00	0.48 a	0.22	0.09	0.51 a
α-glucosidase	0.73 a	0.85a	0.48 8	1.00	0.77ª	0,51 a	0,77ª
β- glucosidase	0.70ª	0.75ª	0.22	0.778	1.00	0.81a	0.74 a
α- galactosidase	0.69°	0.61 a	60:0	0.51ª	0.81 a	1.00	0.52 4
N. acetylgalactosami ne-4-sulphatase	0.68 8	0.88 a	0.51 в	0.77 a	0.74ª	0.52 в	1.00

Figure 25

. PK0.01

26/34

												<u>'</u>	_	•								 -	
N-acetyl galactosamine-4- sulphatase	ng/mL	1.4	0.4	6.0	2.3		1.95	1.32	0.57 ^d	0.53d	1.14	2,28°	_p 00.0	0.00 ^d	5.10°	9.91°	1.98	2.10	1.47	1.41	1.28	1.06	1.67
α-galactosidase	ng/mL	5.2	1.7	2.7	8.5		0.004	0.00 ^d	0.004	1.87d	5.97	6.99	3,98	3.43	3.48	6.74	8.48	3.68	3.78	4.03	12,51°	2,12 ^d	8.740
β- glucosidase	Jm/gu	4.1	1.1	3.0	6.4		4.19	4.30	2.76 ^d	1.21d	2.36 ^d	4.04	3.45	1,58 ^d	4.99	8.71c	5.55	2.80d	2.434	3.89	2.65 ^d	3.95	4.46
α- glucosidase	lm/gn	8.3	2.7	4.0	14.0		6.32	9.94	5.92	4.96	6.51	8,21	7.24	4.25	27.76°	36.38°	0.134	0.194	0.15 ^d	0.00⁴	0.00₫	0.07 ^d	0.094
α-iduronidase	ng/mL	7.0	2.2	4.3	11.2		2.85 ^d	7.76	4.03 ^d	. 0.27 ^d	0.00 ^d	8.90	6.41	5.35	59.02°	33.66	8.49	6.54	3.77 ^d	5.42	2.44 ^d	4.43	7.21
Saposin C	Jm/gu	13.6	3.4	8.9	22.3		29.24°	27.34c	8.60d	7.714	11.11	37.13°	11.76	11.66	31.60	74.10°	16.56	12:73	16.99	20.62	10.91	15.93	27.31°
LAMP-1	ng/mL	34.9	3.8	28.7	40.9		35.55	37.48	29.56	30.01	35,74	52.15°	40.74	29.03	44.07°	44.69°	44 830	35.89	36.61	35.30	34.75	34.51	44.09°
Age		33.4	00	22.8	47.2		38.15	34.86	26.95	ΑN	0.77	3.89	4.84	AN	0.94	1.92		39.21	24.40	57.80	10.65	8.35	10.56
	Adult Controla	Average	O.D	Min	Max	Patientb	Fabry	Fabry	Pahry	MPST	MPS I	MPS II	MPS VI	MPS VI	ML IVI	ML II/II	Domne	Pompe	Pompe	Pompe	Рошре	Pompe	Pomne

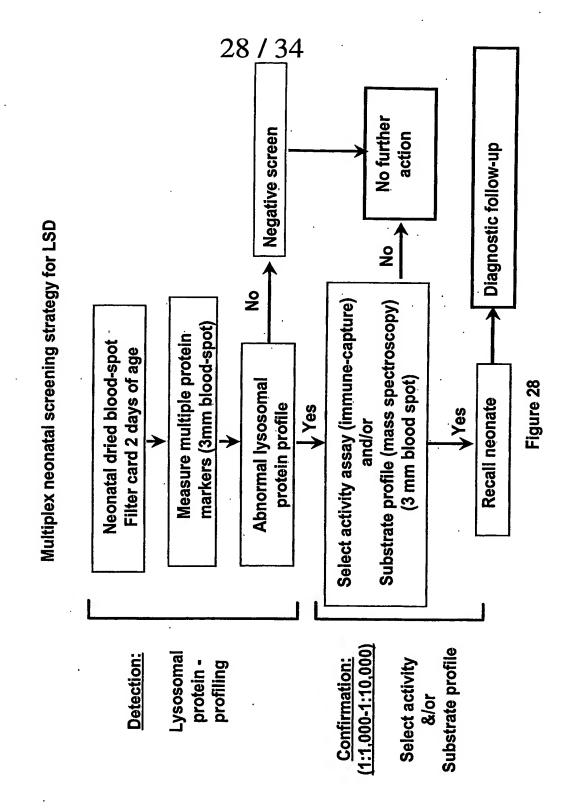
 $^{\rm a}$ Adult controls (n=12); $^{\rm b}$ MPS = mucopolysaccharidosis; ML = mucolipidosis. $^{\rm c}$ indicates above control range; $^{\rm d}$ indicates below control range

27 / 34

	Age	LAMP-1	Saposin C	α- iduronidase	α- glucosidas	β- glucosidase	α- galactosidase	galactosamine-4-
Newborn Controls ^a		ng/mL	ng/mL	ng/mĽ	ngfuL	ng/mL	ng/mL	. ng/mL
Аметаде		58.2	42.9	2.8	9.4	4.6	16.0	2.5
StDev		19.6	22.8	1.2	5.1	2.9	17.3	1.3
Min		19.1	3.7	0.0	1.0	0.8	6.0	0.2
Max		86.5	83.9	5.7	21.0	16.4	72.2	4.8
Patient ^b								
Fabry	38.15	35.55	29.24	2.85	6.32	. 4.19	₽000	1.95
Pabry	34.86	37.48	27.34	291.7	9.94	4.30	0.00 ^d	1.32
Fabry	26.95	29.56	8.60	4,03	5.92	2.76	0.00₫	0.57
MPS I	NA	30.01	7.71	0.27	4.96	1.21	1.87	0.53
MPS I	0.77	35.74	11.11	0.00 ^d	6.51	2.36	. 5.97	1.14
MPS VI	4.84	40.74	.11.76	. 6.41°	7.24	3.45	3.98	0.00 ^d
MPS VI	NA A	29.03	11.66	5.35	4.25	1.58	3.43	0.00 ^d
ML IVIII	. 0.94	44.07	31.60	59.02°	27.76°	4.99	3.48	5.10°
ML II/III	1.92	44.69	74.10	33.66°	36.38°	8.71	6.74	9.91€
Pomne		44.83	16.56	8.49c	. 0,13d	5.55	. 8.48	1.98
Ротре	39.21	35.89	12.73	6.54°	0.194	2.80	3.68	2.10
Pompe	24.40	36.61	16.99	3.77	0.154	2.43	3.78	1.47
Pompe	57.80	35.30	20.62	5.42	0.004	3.89	4.03	1.41
Ротре	10.65	34.75	10.91	2.44	0.00 ^d	2.65	12.51	1.28
Pompe	8.35	34.51	15.93	4.43	0.07 ^d	3.95	2.12	1.06
Pompe	10.56	44.09	27.31	7.21°	0.094	4.46	8.74	1.67
			1	:				

^a Newborn controls (n=28); ^b MPS = mucopolysaccharidosis; ML = mucolipidosis. ^c indicates above control range; ^d indicates below control range

WO 2004/088322 PCT/AU2004/000403

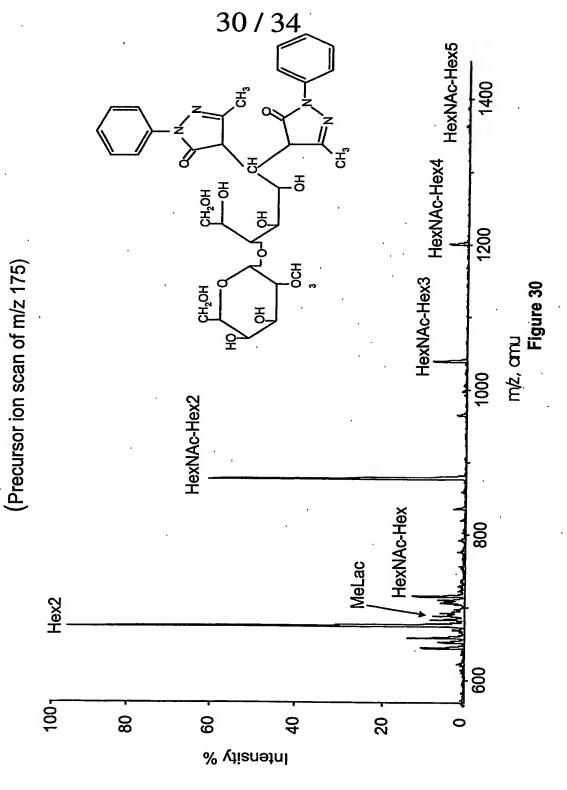


Derivatisation of oligosaccharides for MS/MS

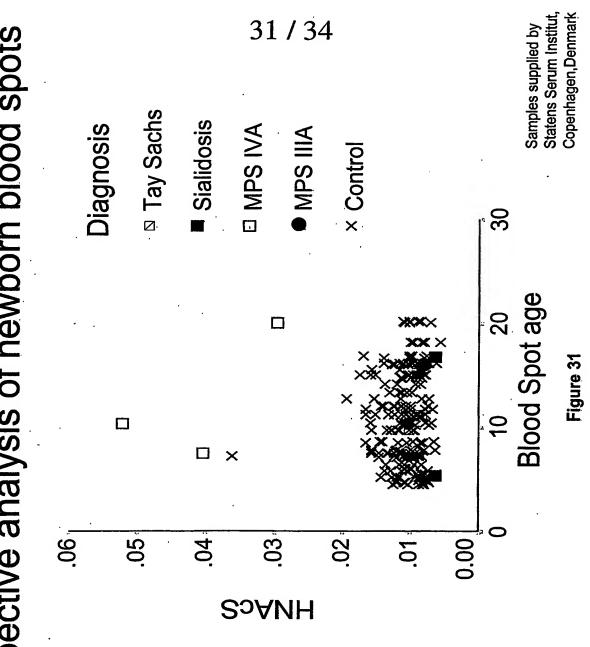
1-phenyl-3-methyl-5-pyrazolone (PMP)

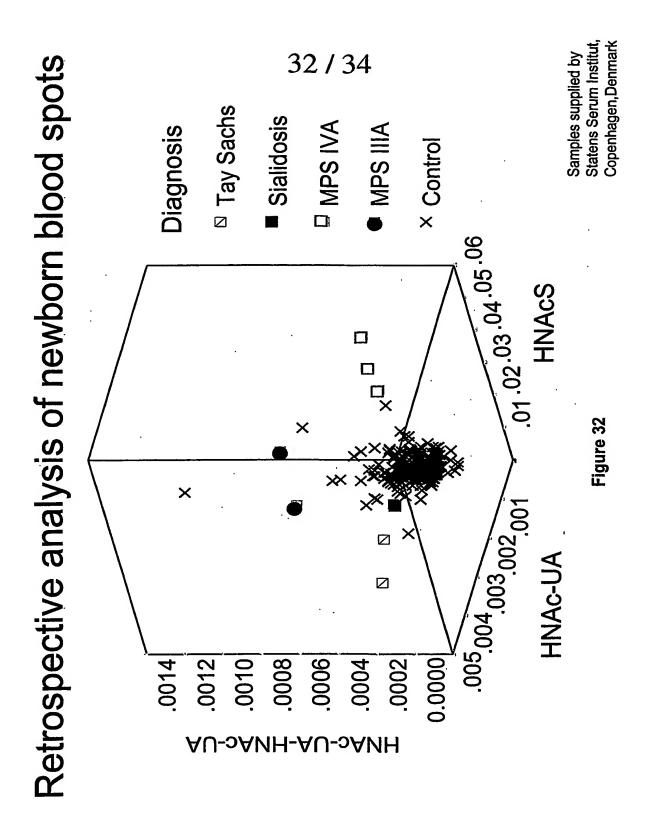
WO 2004/088322 PCT/AU2004/000403

MSMS analysis of α -mannosidosis urine









Summary of retrospective analysis of newborn blood spots

33/34

Disorder	⊆.	Markers	Sensitivity/ Specificity
α-Mannosidosis	~	H2/HNAc	100 / 99.6
MPS II	4	•	•
MPS IIIA	7	HNAc-UA-HNAc-UA	100 / 100
MPS IVA	က	HNAcS	100 / 100
I-cell disease	7	OC/IC	100 / 100
Sialidosis	က	HNS-UA	67 / 100
Pompe disease	က	•	,
Sandhoff disease	9	•	•
Tay-Sachs disease	7	HNAc-UA	100 / 99.6
		•	

Figure 33

34/34

Protei	Protein markers for LSD screening	D screeni	bu
Disorder	Enzyme Deficiency	Australian Prevalence	Therapy
Gaucher disease Fabry disease MPS I Pompe disease MPS VI Krabbe disease MLD MPS IVA Niemann-Pick type A/B MPS IIIA MPS IIIB	β-glucosidase α-galactosidase A α-galactosidase A α-L-iduronidase α-glucosidase N-acetylgalactosamine 4-sulphatase iduronate-2-sulphatase arylsulphatase A galactose 6-sulphatase acid sphingomyelinase heparan-N-sulphatase α-N-acetylglucosaminidase	1 in 57,000 1 in 117,000 1 in 18,000 1 in 146,000 1 in 235,000 1 in 201,000 1 in 201,000 1 in 248,000 1 in 248,000 1 in 114,000	ERT ERT ERT (trials) ERT (trials) BMT BMT BMT BMT BMT RRT (proposed) ERT (proposed) Research Research
TOTAL (n = 12)		1 in 10,500	

Figure 34